

United States Patent

6,541,684

Bowen, et al.

April 1, 2003

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Nucleotide sequences encoding maize RAD51

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**Abstract**

Nucleic acid sequences encoding two RAD51 recombinases active in maize plants are provided. cDNA sequences including the ZmRAD51 coding sequences and unique 3'-untranslated regions which are useful as RFLP probes, are also provided. The production of plasmids containing a nucleic acid sequence encoding a ZmRAD51 fusion protein, as well as the use of the plasmids to introduce the ZmRAD51 coding sequence into a host cell, such as maize cell, are also disclosed.

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Appl. No.: **246963**

Filed: **February 9, 1999**

**Current U.S. Class:** 800/320.1; 435/69.1; 435/196; 536/23.1; 536/23.5;  
536/24.1

**Intern'l Class:** A01H 005/00

**Field of Search:** 536/23.1,23.5,24.1 435/410,468,196,69.1  
800/298,320.1

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*Primary Examiner:* Ketter; James  
*Attorney, Agent or Firm:* Foley & Lardner

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***Parent Case Text***

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**CROSS REFERENCE TO RELATED APPLICATION**

This application claims the benefit of U.S. Provisional Application No. 60/074,745, filed Feb. 13, 1998 and is herein incorporated by reference.

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***Claims***

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What is claimed is:

1. An isolated polynucleotide comprising a member selected from the group consisting of:
  - a) a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 7;
  - b) a polynucleotide having at least 90% identity to a polynucleotide of (a);
  - c) a polynucleotide which will hybridize under ***stringent hybridization*** conditions to said polynucleotide of (a) or (b); and

d) a polynucleotide comprising at least 30 contiguous nucleotides from a polynucleotide of (a), (b) or (c);

wherein the polynucleotide of (a), (b) or (c) encodes a polypeptide with recombinase activity.

2. The isolated polynucleotide of claim 1, wherein said polynucleotide has a sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 6.

3. An expression cassette comprising a polynucleotide of claim 1 operably linked to a promoter.

4. The host cell transfected with an expression cassette of claim 3.

5. The host cell of claim 4, wherein said host cell is a bacterial cell.

6. The host cell of claim 4, wherein said host cell is a sorghum or maize cell.

7. A method of making maize recombinase comprising the steps of:

a) transforming or transfecting a host cell with the expression cassette of claim 3; and

b) purifying the recombinase from the host cell.

8. The method of claim 7, wherein the host cell is selected from the group consisting of a bacterial cell, a plant cell, a mammalian cell and a yeast cell.

9. A method of modulating ZmRAD 51 activity in a plant, comprising:

(a) introducing into a plant cell an expression cassette comprising an isolated polynucleotide of claim 1 operatively linked to a promoter;

(b) culturing the plant cell under plant cell growing conditions;

(c) regenerating a plant which possesses the transformed genotype, and

(d) inducing expression of said polynucleotide for a time sufficient to modulate ZmRAD51 activity in said plant.

10. A transgenic plant cell comprising an isolated polynucleotide of claim 1.

11. A transgenic plant comprising an isolated polynucleotide of claim 1.

12. A transgenic seed from the transgenic plant of claim 11.

13. Primer pairs for isolating at least a part of a Zea mays recombinase gene, selected

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Protein having glutaminase activity and gene encoding the same

**Abstract**

There are disclosed a protein having an amino acid sequence represented by amino acid numbers 1 to 684 or 49 to 684 shown in SEQ ID NO:2, or a protein having a glutaminase activity in which one or more amino acids is/are deleted from, substituted by, inserted to or added to the amino acid sequence of the above protein; a gene containing DNA encoding the above protein or a gene encoding a protein which hybridizes with the DNA of the above gene under a stringent condition and has a glutaminase activity; a recombinant DNA containing the above gene; a transformant or a transductant containing the above recombinant DNA; and a process for producing glutaminase which comprises culturing the above transformant or the above transductant and collecting glutaminase from a culture medium.

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Assignee: **Kikkoman Corporation** (Chiba-ken, JP)

Appl. No.: **946678**

Filed: **September 6, 2001**

**Foreign Application Priority Data**

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Sep 06, 2000[JP]	2000-270371
<b>Current U.S. Class:</b>	<b>435/227</b> ; 435/320.1; 435/223.2; 435/440; 435/6; 435/252.3; 536/23.2
<b>Intern'l Class:</b>	C12N 009/78; C12N 015/00; C12N 001/20; C07H 021/04
<b>Field of Search:</b>	435/227,320.1,257.3,232.2,440,6,272.2 536/23.2

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<b>Foreign Patent Documents</b>			
A11332553	Dec., 1999	JP.	

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*Primary Examiner:* Achutamurthy; Ponnathapu

*Assistant Examiner:* Pak; Yong

*Attorney, Agent or Firm:* Birch, Stewart, Kolasch & Birch, LLP

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***Claims***

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What is claimed is:

1. An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding an amino acid sequence comprising amino acids 1 to 634 of SEQ ID NO:2, and

(b) a nucleotide sequence, the complement thereof which binds to said nucleotide sequence (a) under ***stringent hybridization*** conditions of a sodium concentration of 50-300 mM and a temperature of 42-68.degree. C., wherein said nucleic acid encodes a protein having glutaminase activity.

2. An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding an amino acid sequence comprising amino acids 49 to 684 of SEQ ID NO:2, and

(b) a nucleotide sequence, the complete complement thereof which binds to said nucleotide sequence (a) under ***stringent hybridization*** conditions of a sodium concentration of 50-300 mM and a temperature of 42-68.degree. C.,

wherein said nucleic acid encodes a protein having glutaminase activity.

3. An isolated nucleic acid comprising a nucleotide sequence of SEQ ID NO:1, or a complete complement thereof which binds to SEQ ID NO:1 under ***stringent hybridization*** conditions of a sodium concentration of 50-300 mM and a temperature of 42-68.degree. C.,

wherein said nucleic acid encodes a protein having glutaminase activity.

4. A recombinant nucleic acid comprising the nucleotide sequence of claim 3.

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Nucleic acid encoding PTH1R receptor

**Abstract**

The present invention relates to novel parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP) receptors (PTH1R and PTH3R) isolated from zebrafish. The receptors of the present invention share homology with previously identified parathyroid hormone (PTH)/parathyroid related protein (PTHrP) receptors. Isolated nucleic acid molecules are provided encoding the zebrafish PTH1R and PTH3R receptors. PTH1R and PTH3R receptor polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of PTH1R and PTH3R receptor activity and to diagnostic and therapeutic methods.

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Assignee: **The General Hospital Corporation** (Boston, MA)

Appl. No.: **449632**

Filed: **November 30, 1999**

**Current U.S. Class:** 435/69.1; 536/23.5; 536/24.3; 536/24.31; 530/350;  
435/71.1; 435/71.2; 435/471; 435/325; 435/320.1;  
435/252.3; 435/254.11

**Intern'l Class:** C12N 015/12; C12N 015/63; C12N 005/10; C07K  
014/705

**Field of Search:** 536/23.1,23.5,24.3,24.31 530/350  
435/69.1,71.1,71.2,471,325,252.3,254.11,320.1

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<u>5496801</u>	Mar., 1996	Holthuis et al.	514/12.
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<u>5693616</u>	Dec., 1997	Krstenaansky et al.	514/12.
<u>5695955</u>	Dec., 1997	Krstnenansky et al.	435/69.
<u>5798225</u>	Aug., 1998	Krstenansky et al.	435/69.
<u>5814603</u>	Sep., 1998	Oldenburg et al.	514/17.

Terminus Limit the Response of the Parathyroid Hormone (PTH) 2 Receptor to PTH-related Peptide," J. Biol. Chem. 273:3830-3837 (Feb. 1998).  
International Search Report for International Application No. PCT/US99/28207,  
mailed Apr. 18, 2000.

*Primary Examiner:* Mertz; Prema

*Attorney, Agent or Firm:* Sterne, Kessler, Goldstein & Fox PLLC

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***Government Interests***

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**STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY-  
SPONSORED RESEARCH AND DEVELOPMENT**

Part of the work performed during development of this invention utilized U.S.  
Government funds. The U.S. Government has certain rights in this invention.

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***Parent Case Text***

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This application claims the benefit of the filing date of provisional application No.  
60/110,467 filed on Nov. 30, 1998, which is herein incorporated by reference.

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***Claims***

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What is claimed is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide  
sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a parathyroid hormone (PTH)/PTH-related peptide  
(PfhRrP) receptor (PTH1R receptor) having the complete amino acid sequence of amino  
acids 1 to 536 in SEQ ID NO:2;

(b) a nucleotide sequence encoding the PTH1R receptor having the amino acid sequence  
of amino acids 2 to 536 in SEQ ID NO:2;

(c) a nucleotide sequence encoding the mature PTH1R receptor having the amino acid  
sequence of amino acids 25 to 536 in SEQ ID NO:2;

(d) a nucleotide sequence encoding the PTH1R receptor having the complete amino acid  
sequence encoded by the cDNA clone deposited with the ATCC as deposit PTA-916;

(e) a nucleotide sequence encoding the mature PTH1R receptor having the amino acid sequence encoded by the cDNA deposited with the ATCC as deposit PTA-916;

(f) a nucleotide sequence encoding the PTH1R extracellular domain, said extracellular domain having the amino acid sequence of amino acids 25 to 147 in SEQ ID NO:2;

(g) a nucleotide sequence encoding the PTH1R transmembrane domain, said transmembrane domain having the amino acid sequence of amino acids 148 to 416 in SEQ ID NO:2; and

(h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g).

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone deposited with the ATCC as deposit PTA-916.

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the PTH1R receptor having the complete amino acid sequence encoded by the cDNA deposited with the ATCC as deposit PTA-916.

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the mature PTH1R receptor having the amino acid sequence encoded by the cDNA clone deposited with the ATCC as deposit PTA-916.

5. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under **stringent hybridization** conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f), (g) or (h) of claim 1;

wherein said **stringent hybridization** conditions consist of overnight incubation at 42.degree. C. in a solution comprising: 50% formamide, 5.times.SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5.times.Dehardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1.times.SSC at 65.degree. C.;

wherein said polynucleotide which hybridizes does not have the nucleotide sequence of a human, mouse, rat or bovine PTH1R;

and wherein said polynucleotide which hybridizes does not hybridize under **stringent hybridization** conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

6. An isolated nucleic acid molecule comprising a polynucleotide which encodes an epitope-bearing region of a PTH1R receptor comprising an amino acid sequence of between 7 and 30 amino acids from the amino acid sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1.



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Motor proteins and methods for their use

**Abstract**

The invention provides isolated nucleic acid and amino acid sequences of HsKip3d, antibodies to HsKip3d, methods of screening for HsKip3d modulators using biologically active HsKip3d, and kits for screening for HsKip3d modulators.

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Appl. No.: **632344**

Filed: **August 3, 2000**

**Current U.S. Class:** 435/320.1; 435/183; 435/189; 435/252.3;  
435/254.11; 435/257.2; 435/325; 536/23.2

**Intern'l Class:** C07H 021/04; C12N 015/00; C12N 005/00; C12N  
001/20

**Field of Search:** 536/23.1,23.5,23.2  
435/320.1,325,189,183,252.3,254.11,257.2

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Stewart et al. (1997) Genbank Accession #U89264. Direct Submission, Dm Kinesin-like protein 67a.

*Primary Examiner:* Duffy; Patricia A.

*Attorney, Agent or Firm:* Stevens; Lauren L. Beyer Weaver & Thomas, LLP

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***Claims***

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What is claimed is:

1. An isolated nucleic acid sequence encoding a motor protein, wherein the motor protein has the following properties: (i) the protein has microtubule stimulated ATPase activity; and (ii) the protein comprises a sequence that has greater than 90% sequence identity to SEQ ID NO:2 or SEQ ID NO:4 as measured using a sequence comparison algorithm.
2. An isolated nucleic acid sequence of claim 1, wherein the protein specifically binds to polyclonal antibodies to a protein comprising SEQ ID NO:2 or SEQ ID NO:4.
3. An isolated nucleic acid sequence, wherein the nucleic acid encodes a protein having an amino acid sequence comprising SEQ ID NO:2 or SEQ ID NO:4.
4. An isolated nucleic acid sequence having a nucleotide sequence comprising SEQ ID NO:1 or SEQ ID NO:3.
5. An isolated nucleic acid sequence of claim 1, wherein the nucleic acid sequence selectively hybridizes under high stringency conditions to a nucleic acid having a sequence or complementary sequence of SEQ ID NO:1 or SEQ ID NO:3, wherein said ***stringent hybridization*** conditions are selected from the group consisting of (1) 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50.degree. C.; (2) 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride and 75 mM sodium citrate at 42.degree. C.; and (3) 50% formamide, 5.times.SSC (0.75 M sodium chloride, 0.75 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5.times.Denhardt's solution, 50 .mu.g/mL sonicated salmon sperm DNA, 0.1% sodium dodecyl sulfate and 10% dextran sulfate at 42.degree. C., with washes at 42.degree. C. in 0.2.times.SSC and 50% formamide at 55.degree. C., followed by a wash of 0.1.times.SSC containing EDTA at 55.degree. C.
6. An expression vector comprising a nucleic acid encoding a motor protein, wherein the motor protein has the following properties: (i) the protein has microtubule stimulated ATPase activity; and (ii) the protein comprises a sequence that has greater than 90% sequence identity to SEQ ID NO:2 or SEQ ID NO:4 as measured using a sequence comparison algorithm.

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Gene encoding hyaluronan synthase

**Abstract**

An isolated and purified DNA molecule encoding hyaluronan synthase-2 (Has2) is provided, as is purified and isolated Has2 polypeptide.

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Inventors: **McDonald; John A.** (Scottsdale, AZ); **Spicer; Andrew P.** (Scottsdale, AZ);  
**Augustine; Mary Louise** (Scottsdale, AZ)

Assignee: **Clear Colutions Biotech, Inc.** (Stony Brook, NY)

Appl. No.: **675499**

Filed: **July 3, 1996**

**Current U.S. Class:** 435/183; 435/84; 435/252.3; 435/320.1; 435/325;  
536/23.1; 536/23.2

**Intern'l Class:** C12N 009/00; C07H 021/04

**Field of Search:** 435/183,240.2,252.3,320.1,325,84 536/23.2,23.1  
935/22

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*Primary Examiner:* Prouty; Rebecca E.

*Assistant Examiner:* Hutson; Richard

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### *Claims*

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What is claimed is:

1. An isolated and purified DNA molecule comprising a DNA segment encoding a

mammalian hyaluronan synthase or an enzymatically active fragment thereof, wherein the DNA molecule hybridizes under *stringent hybridization* conditions to the DNA molecule having SEQ ID NO:1 or the complement thereof.

2. An expression cassette comprising a promoter operably linked to the DNA molecule of claim 1.

3. A host cell transformed with the DNA molecule of claim 1.

4. A method to produce hyaluronan synthase, comprising: culturing a host cell transformed with the DNA molecule of claim 1 operably linked to a promoter, so that said host cell expresses recombinant hyaluronan synthase.

5. The method of claim 4 further comprising isolating said hyaluronan synthase from the host cell.

6. A method of altering the amount of hyaluronan produced by a cell, comprising:

(a) introducing into a host cell the DNA molecule of claim 1 operably linked to a promoter functional in the host cell so as to yield a transformed host cell; and

(b) expressing the DNA segment in the transformed host cell in an amount that alters the amount of hyaluronan produced by the transformed cell relative to the amount of hyaluronan produced by a corresponding untransformed cell.

7. The method of claim 6 wherein the amount of hyaluronan produced by the transformed host cell is increased relative to the amount of hyaluronan produced by the corresponding untransformed host cell.

8. The method of claim 6 wherein the amount of hyaluronan produced by the transformed host cell is decreased relative to the amount of hyaluronan produced by the corresponding untransformed host cell.

9. The DNA molecule of claim 1 wherein the DNA segment encodes murine hyaluronan synthase.

10. The DNA molecule of claim 9 or 1 wherein the DNA segment encodes the hyaluronan synthase having SEQ ID NO:2.

11. The DNA molecule of claim 1 wherein the DNA segment comprises SEQ ID NO:1.

12. The DNA molecule of claim 1 wherein the DNA segment encodes human hyaluronan synthase.

13. The DNA molecule of claim 12 or 1 wherein the DNA segment comprises SEQ ID NO:23.

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Nucleic acid molecules encoding CASPR/p190

**Abstract**

The 190 kDa Contactin ASSociated PRotein (CASPR/p190) is identified and is implicated as the bridge between contactin and intracellular second messenger systems for the signal caused by the binding of the carboxy anhydrase domain of RPTP.beta. to contactin and resulting in neurite growth, differentiation or survival. Mammalian CASPR/p190 cDNAs and proteins are described, including those from human and rat. In addition, particular domains of the proteins are characterized.

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Inventors: **Peles; Elior** (Foster City, CA)

Assignee: **Sugen, Inc.** (South San Francisco, CA)

Appl. No.: **826134**

Filed: **March 26, 1997**

**Current U.S. Class:** 435/69.1; 435/70.1; 435/71.1; 435/71.2; 435/243;  
435/252.3; 435/254.2; 435/320.1; 435/325; 536/23.5

**Intern'l Class:** C07H 021/04; C12N 015/00; C12N 001/20; C12P  
021/04

**Field of Search:** 536/23.1,23.5  
435/69.1,70.1,71.1,71.2,325,243,252.3,320.1,254.2

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*Primary Examiner:* Duffy; Patricia A.

*Attorney, Agent or Firm:* Foley & Lardner

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***Parent Case Text***

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The present application claims priority under 35 U.S. .sctn. 119(e) to provisional application Ser. No. 60/014,199, filed Mar. 27, 1996, the entire contents of which is incorporated herein by reference in its entirety.

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***Claims***

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What is claimed is:

1. An isolated nucleic acid molecule comprising a nucleotide sequence that:
  - (a) encodes the amino acid sequence SEQ ID NO:2; or
  - (b) encodes the amino acid sequence SEQ ID NO:4.
2. An isolated nucleic acid molecule comprising a nucleotide sequence that hybridizes under highly stringent conditions to the full complement of the nucleic acid molecule of claim 1, and encodes a naturally occurring p190 polypeptide, wherein the highly ***stringent hybridization*** conditions comprise washing in 0.1.times.SSC/0.1% SDS at 68.degree. C.
3. An isolated nucleic acid molecule which comprises (a) a nucleotide sequence that encodes a polypeptide having the amino acid sequence shown in SEQ ID NO:2 from amino acid residues 40-168, 199-330, 362-486, 544-576, 582-739, 809-938, 961-985, 1031-1077, 1083-1218, or 1328-1369; or (b) a nucleotide sequence that is fully complementary to the nucleotide sequence of (a).
4. An isolated nucleic acid molecule which comprises
  - (a) a nucleotide sequence that encodes a p190 polypeptide lacking at least one, but not all, of the following segments of amino acid residues: 40-168, 199-330, 362-486, 544-576, 582-739, 809-938, 961-985, 1031-1077, 1083-1218, 1282-1306, or 1328-1369 of SEQ ID NO:2; or;

**United States Patent**  
**De Villiers-Zur Hausen , et al.**

**6,413,522**  
**July 2, 2002**

**Papilloma viruses, products for the detection thereof as well as for treating diseases caused by them**

### **Abstract**

This invention relates to a DNA coding for a peptide of a papilloma virus major capsid protein and a papilloma virus genome, respectively. Furthermore, this invention concerns proteins coded by the papilloma virus genome and antibodies directed thereagainst as well as the use thereof for diagnosis, treatment and vaccination.

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**Assignee:** Deutsches Krebsforschungszentrum Stiftung des Offentlichen Rechts (Heidelberg, DE)

**Appl. No.:** 319056

**Filed:** August 5, 1999

**PCT Filed:** November 12, 1997

**PCT NO:** PCT/DE97/02659

**371 Date:** August 5, 1999

**102(e) Date:** August 5, 1999

**PCT PUB.NO.:** WO98/23752

**PCT PUB. Date:** June 4, 1998

### **Foreign Application Priority Data**

Nov 26, 1996[DE]

196 48 962

**Current U.S. Class:** 424/204.1; 424/199.1; 435/5; 435/69.1; 435/69.3; 435/235.1; 435/320.1; 536/23.72; 530/350; 530/324

**Intern'l Class:** A61K 039/12; C12N 001/12; C12N 015/00; C07H 021/04

**Field of Search:** 435/6,701,69.3,69.1,235.1,320.1 536/23.1,23.72 396/2,1 530/350,387.1,324 424/204.1,199.1

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*Primary Examiner:* Salimi; Ali R.

*Attorney, Agent or Firm:* Pennie & Edmonds LLP

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***Parent Case Text***

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This is a national phase filing of the Application No. PCT/DE97/02659, which was filed with the Patent Corporation Treaty on Nov. 12, 1997, and is entitled to priority of the German Patent Application DE 196 48 962.8, filed Nov. 26, 1996.

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***Claims***

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What is claimed is:

1. An isolated polynucleotide consisting essentially of:

(a) a nucleotide sequence encoding the peptide of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8;

(b) a nucleotide sequence hybridizing to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7; or

(c) the complement of (a) or (b);

wherein the polynucleotide has a homology of at least 90% to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, or the complement thereof.

2. An isolated polynucleotide encoding a peptide of a papilloma virus major capsid protein, wherein the said polynucleotide has been obtained using the following steps:

(a) incubating total DNA isolated from a biopsy of epithelial neoplasm with a nucleic acid having at least a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, under a condition that allows hybridization of a polynucleotide derived from a papilloma virus genome included in the total DNA to said nucleotide sequence of the complement of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7; and

(b) identifying and isolating a polynucleotide that hybridizes to the complement of the nucleotide

sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7 in step (a);

wherein the polynucleotide has a homology of at least 90% to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7.

3. An isolated polynucleotide, consisting essentially of (a) a nucleic acid encoding a peptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8, or (b) the complement of (a).

4. An isolated polynucleotide, wherein the polynucleotide consists essentially of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, or the complement thereof.

5. A plasmid comprising the polynucleotide of claim 1 or 2.

6. A plasmid comprising the polynucleotide of claim 3 or 4.

7. An expression vector comprising the polynucleotide of claim 1 or 2.

8. An expression vector comprising the polynucleotide of claim 3 or 4.

9. A host cell comprising the plasmid of claim 5.

10. A host cell comprising the plasmid of claim 6.

11. A host cell comprising the expression vector of claim 9.

12. A host cell comprising the expression vector of claim 8.

13. A method of producing a peptide of a papilloma virus major capsid protein, comprising cultivating the host cell of claim 11 under suitable conditions.

14. A method of producing a peptide of a papilloma virus major capsid protein, comprising cultivating the host cell of claim 12 under suitable conditions.

15. A method of detecting a papilloma virus DNA, comprising:

(a) **hybridizing under stringent conditions** at least a portion of the polynucleotide of claim 1, 2, 3, or 4 to a DNA sample; and

(b) identifying papilloma virus in said DNA sample by detecting a hybridization signal.

16. A composition comprising the polynucleotide of claim 1, 2, 3, or 4 as reagent for diagnosis and a diagnostically acceptable carrier.

17. A method of producing a papilloma virus genome, comprising:

(a) incubating total DNA isolated from a biopsy of epithelial neoplasm with a nucleic acid having at least a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, under a condition that allows hybridization of a polynucleotide derived from a papilloma virus genome included in the total DNA to said nucleotide sequence of the complement of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7; and

**United States Patent**  
**Quandt , et al.**

**6,384,304**  
**May 7, 2002**

## Conditional sterility in wheat

### Abstract

The invention relates to the use of a deacetylase coding sequence for obtaining conditional sterility in wheat. The invention relates to vectors comprising a deacetylase coding sequence under control of promoters which direct stamen-selective expression in wheat, which are particularly suited for the production of wheat plants which can be made male-sterile upon application of an acetylated toxin.

**Inventors:** **Quandt; Jurgen** (Saskatoon, CA); **Bartsch; Klaus** (Konigstein, DE); **Knittel; Nathalie** (Frankfurt, DE)

**Assignee:** **Plant Genetic Systems N.V.** (Gent, BE)

**Appl. No.:** **418817**

**Filed:** **October 15, 1999**

**Current U.S. Class:** **800/320.3; 435/418; 435/419; 435/468; 800/271; 800/274; 800/278; 800/287; 800/288; 800/300; 800/303**

**Intern'l Class:** **C12N 015/82; C12N 015/31; C12N 005/04; A01H 005/00; A01H 001/02**

**Field of Search:** **800/320.3,288,300,287,274,303,271,278 435/418,419,468**

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<u>5767371</u>	Jun., 1998	Broer et al.	800/205.
<u>5767374</u>	Jun., 1998	DeGreef et al.	800/205.
<u>5792929</u>	Aug., 1998	Mariani et al.	800/205.
<u>5981189</u>	Nov., 1999	Chan et al.	435/6.
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"The development of a nuclear male sterility system in wheat. Expression of the barnase gene under the control of tapetum specific promoters"; DeBlock et al., 1997, Theor. Genet. 95: 125-131.

*Primary Examiner:* Fox; David T.

*Attorney, Agent or Firm:* Frommer Lawrence & Haug LLP

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### Claims

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We claim:

1. A wheat plant, having a chimeric gene integrated into its genome, the chimeric gene comprising:

a) a DNA molecule encoding a deacetylase from *Stenotrophomonas* sp; and

b) a promoter directing stamen-selective expression in wheat wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under control of the stamen-selective promoter.

2. The wheat plant of claim 1, wherein said DNA molecule encodes a biologically active fragment or a variant of the deacetylase encoded by SEQ ID No. 9.

3. The wheat plant of claim 1, wherein said DNA molecule encodes a deacetylase having the amino acid sequence of SEQ ID No. 8.

4. The wheat plant of claim 1, wherein said DNA molecule comprises the sequence of SEQ ID No. 9, or a sequence capable of hybridizing to SEQ ID No. 9 under standard *stringent* conditions.

5. The wheat plant of claim 1, wherein said stamen-selective promoter is a CA55 promoter.

6. The wheat plant of claim 1, wherein said stamen-selective promoter is a T72 promoter.

7. The wheat plant of claim 1, wherein said stamen-selective promoter is an E1 promoter.

8. A process for producing *hybrid* wheat seed, said process comprising

i) producing seeds capable of growing into conditionally male-sterile wheat plants, said seeds having a chimeric gene integrated in their genome, the chimeric gene comprising:

a) a DNA molecule encoding a deacetylase from *Stenotrophomonas* sp., and

b) a stamen-selective promoter wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under the control of the stamen-selective promoter;

- ii) interplanting said seeds capable of growing into conditionally male-sterile wheat plants with seeds capable of growing into male fertile wheat plants;
- iii) inducing male-sterility in said conditionally male-sterile plants by applying an N-acetyl-PPT, which in itself is not toxic to the plants or plant cells; and
- iv) harvesting *hybrid* seed.

9. The process of claim 8, wherein said DNA molecule encodes a biologically active fragment or a variant of the deacetylase encoded by SEQ. ID. No. 9.

10. The process of claim 8, wherein said DNA molecule encodes the deacetylase comprising the amino acid sequence of SEQ ID No. 8.

11. The process of claim 8, wherein said DNA molecule comprises the sequence of SEQ ID No. 9, or a sequence capable of hybridizing to SEQ ID No. 9 under standard *stringent* conditions.

12. The process of claim 8, wherein said stamen-selective promoter is a CA55 promoter.

13. The process of claim 8, wherein said stamen-selective promoter is a T72 promoter.

14. The process of claim 8, wherein said stamen-selective promoter is an E1 promoter.

15. The process of claim 8, wherein said male fertile plants are female-sterile.

16. A process for producing a conditionally male-sterile wheat plant, said process comprising

i) transforming a wheat plant cell or tissue with a chimeric gene which comprises:

a) a DNA molecule encoding a deacetylase from *Stenotrophomonas* sp., and

b) a stamen-selective promoter wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under the control of said stamen-selective promoter;

ii) regenerating said conditionally male-sterile plant from said cell or tissue; and optionally,

iii) applying an N-acetyl-PPT to said conditionally male-sterile plant, which is in itself not toxic to the plant or plant cells to make said plant male-sterile.

17. The process of claim 16, wherein said DNA molecule encodes a biologically active fragment of the deacetylase encoded by SEQ. ID. No. 9.

18. The process of claim 16, wherein said DNA molecule encodes the deacetylase of SEQ ID No. 8.

19. The process of claim 16, wherein said DNA molecule comprises the sequence of SEQ ID No. 9, or a sequence capable of hybridizing to SEQ ID No. 9 under standard *stringent* conditions.

20. The process of claim 16, wherein said stamen-selective promoter is a CA55 promoter.

21. The process of claim 16, wherein said stamen-selective promoter is a T72 promoter.

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Seed specific promoters

**Abstract**

The present invention is directed to isolated promoter sequences from seed-specific genes, such as KNAT411. When operably linked to either the coding sequence of a heterologous gene or a sequence complementary to a native plant gene, the subject promoters direct expression of the coding sequence or complementary sequence in a plant seed, including the early embryo. The promoter sequences are useful in expression cassettes and expression vectors for the transformation of plants. Also provided are methods of directing seed-specific expression of a gene or sequence complementary to a native plant gene by introducing into a plant cell an isolated nucleic acid comprising a subject promoter operably linked to said gene or complementary sequence. Methods for activating a site-specific recombination system in the early embryo of a seed by transforming a plant with an expression cassette comprising a subject promoter operably linked to a recombinase gene are also provided.

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Inventors: **Thomas; Terry L.** (College Station, TX); **Hsieh; Tzung-Fu** (College Station, TX)

Assignee: **Rhone-Poulenc Agro** (Lyons, FR)

Appl. No.: **306060**

Filed: **May 6, 1999**

**Current U.S. Class:** 800/287; 435/69.1; 435/320.1; 435/419; 435/468; 435/471; 536/24.1; 800/281; 800/298; 800/306; 800/312; 800/314; 800/320.1; 800/322

**Intern'l Class:** C12N 005/04; C12N 015/82; C12N 015/90; A01H 005/00; A01H 005/10

**Field of Search:** 435/69.1,320.1,410,412,415,416,414,419,468 536/23.6,24.1 800/278,281,286,287,295,298,306,312,314,317.3,320.1,322

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*Primary Examiner:* Benzion; Gary

*Assistant Examiner:* Mehta; Ashwin

*Attorney, Agent or Firm:* Scully, Scott, Murphy & Presser

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### *Claims*

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What is claimed is:

1. An isolated nucleic acid comprising a promoter which has the seed-specific promoter activity of the KNAT411 promoter, said promoter hybridizing under ***stringent hybridization*** conditions to the KNAT411 promoter having the nucleotide sequence as set forth in SEQ ID NO:1, wherein said ***stringent hybridization*** conditions comprise hybridization in 4.times.SSC at 65.degree. C. and washing in 0.1.times.SSC at 65.degree. C.
2. An isolated nucleic acid comprising a promoter which has the seed-specific promoter activity of the KNAT411 promoter, said promoter having a sequence identity of about 60% to about 65% when compared to the nucleotide sequence of the KNAT411 promoter as set forth in SEQ ID NO:1.
3. An isolated nucleic acid comprising a promoter which has the seed-specific promoter activity of the KNAT411 promoter, said promoter having a sequence identity of about 65% to about 75% when compared to the nucleotide sequence of the KNAT411 promoter as set forth in SEQ ID NO:1.
4. An isolated nucleic acid comprising a promoter which has the seed-specific promoter activity of the KNAT411 promoter, said promoter having a sequence identity of about 75% to about 85% when compared to the nucleotide sequence of the KNAT411 promoter as set forth in SEQ ID NO:1.
5. An isolated nucleic acid comprising a promoter which has the seed-specific promoter

United States Patent  
Kunst, et al.

6,274,790  
August 14, 2001

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Nucleic acids encoding a plant enzyme involved in very long chain fatty acid synthesis

**Abstract**

Nucleic acid molecules encoding an enzyme involved in very long chain fatty acid (VLCFA) elongation in plants are disclosed. The invention includes a cDNA, genomic clone and encoded protein, as well as plants having modified VLCFA composition, such as modified epicuticular waxes, and methods of making such plants.

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Inventors: **Kunst; Ljerka** (North Vancouver, CA); **Millar; Anthony A.** (Vancouver, CA)

Assignee: **The University of British Columbia** (Vancouver, CA)

Appl. No.: **058947**

Filed: **April 10, 1998**

**Current U.S. Class:** 800/287; 435/468; 536/24.1; 800/281; 800/298

**Intern'l Class:** C12N 015/82; A01H 005/00; C07H 021/04

**Field of Search:** 800/298,281,264,287 435/69.1,468,419,430,320.1 536/23.6

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*Primary Examiner:* McElwain; Elizabeth F.

*Attorney, Agent or Firm:* Klarquist Sparkman Campbell Leigh & Whinston, LLP

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***Parent Case Text***

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**PRIORITY CLAIM**

This application claims priority to co-pending U.S. provisional patent application Ser. No. 60/043,831, filed on Apr. 14, 1997.

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***Claims***

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We claim:

1. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleic acid sequence, wherein the promoter sequence comprises a transcriptional regulatory region capable of mediating gene expression in epidermal cells of Arabidopsis wherein the transcriptional regulatory region hybridizes under stringent conditions to: Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.
2. A recombinant nucleic acid molecule according to claim 1 wherein the promoter sequence comprises at least 50 consecutive nucleotides of the sequence shown in Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.
3. The recombinant nucleic acid molecule according to claim 1, wherein the promoter sequence is at least 70% identical to the sequence set forth in Seq. I.D. No. 12.
4. A recombinant nucleic acid molecule according to claim 1, wherein the promoter sequence is at least 80% identical with the sequence set forth in Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.

5. A recombinant vector comprising a nucleic acid molecule according to claim 1.
6. A transgenic plant comprising a heterologous nucleic acid sequence, wherein the heterologous nucleic acid sequence comprises the recombinant nucleic acid molecule of claim 1.
7. The recombinant nucleic acid molecule according to claim 1, wherein the nucleic acid sequence encodes a protein having very long chain fatty acid elongase activity.
8. A method of producing a transgenic plant comprising introducing into the plant the recombinant nucleic acid molecule of claim 1.
9. A plant produced by sexual or asexual propagation of the transgenic plant produced according to the method of claim 8, or by propagation of progeny of the transgenic plant, wherein the plant comprises the recombinant nucleic acid molecule.
10. A method of isolating a nucleic acid molecule having promoter activity, comprising **hybridizing under stringent conditions** a nucleic acid preparation with a probe comprising Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.
11. A plant cell comprising a heterologous nucleic acid sequence, wherein the heterologous nucleic acid sequence comprises the recombinant nucleic acid molecule of claim 1.
12. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleic acid sequence, wherein the promoter sequence comprises a transcriptional regulatory region capable of mediating gene expression in epidermal cells of Arabidopsis wherein the transcriptional regulatory region is obtainable from a plant VLCFA condensing enzyme gene comprising an open reading frame that hybridizes under stringent conditions to Seq. I.D. No. 3 or to the complement of Seq. I.D. No. 3.